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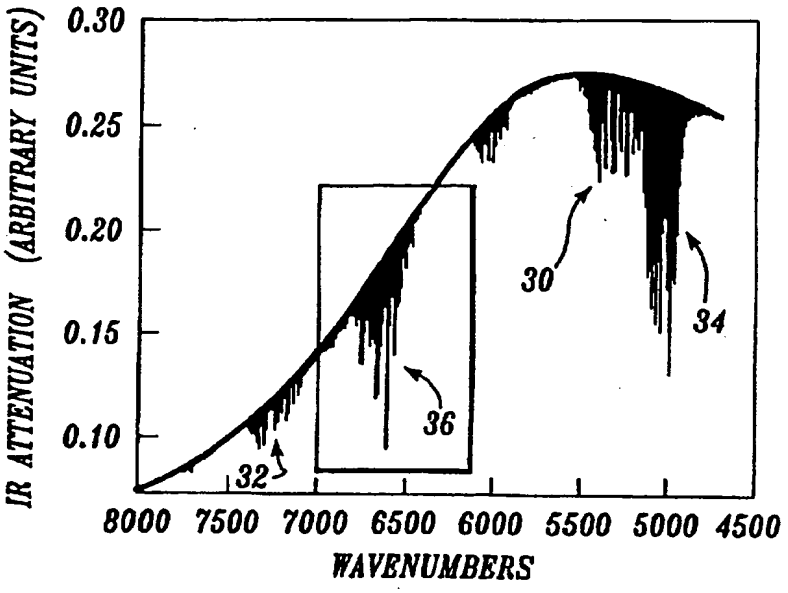
(54) Title: OPTICAL NON-RADIOACTIVE BREATH ANALYSIS

(57) Abstract

The invention is a method of measuring ammonia in a breath sample with a room temperature, near infrared laser. The invention is particularly useful for indicating the presence and activity of an intragastrointestinal *Helicobacter pylori* or other ammonia compound producing metabolisis.



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OPTICAL NON-RADIOACTIVE BREATH ANALYSIS

FIELD OF THE INVENTION

The present invention relates generally to a method for breath analysis using non-radioactive biomarkers that are optically quantified. More specifically, the invention is optical quantification of non-radioactive biomarkers in a rough vacuum with infrared light. In this patent application, the word breath is a vapor that includes both exhaled air from the lungs or perspiration vapor or sweat vapor transpired through the skin.

BACKGROUND OF THE INVENTION

The diagnosis of pathologies and disease by analysis of exhaled air has been postulated since the time of Hypocrites (*ca.*, 400 BC).[1] It is a well established fact that a number of pathologies are associated with the presence of distinct endogenous volatile species in the breath. For instance, both diabetes mellitus and pancreatitis, when left untreated have been associated with the production of relatively large amounts of ketones (acetone).[2,3] Unusually high levels of acetone are also indicative of dietary imbalance.[4] Then acetone can be readily detected by its characteristic sweet odor in the breath. Methane and hydrogen are indicative of intestinal disorders.[5] Hydrogen peroxide is indicative of impaired pulmonary function.[6] Short alkanes such as pentane and ethane are associated with *in vivo* lipid oxidation.[7] Excessive levels of carbon monoxide may be indicative of a malfunction in heme production/breakdown.[8] Carbonyls such as formaldehyde and acetaldehyde have been seen in tumor bearing mice.[9] The combination of finding several species simultaneously (*i.e.*, acetone, methyl ethyl ketone and *n*-propanol) has been statistically correlated with lung cancer in humans.[10] It is further known that analysis of perspiration can be used for indication of state of health or

presence of a disease (U.S. patent 5,465,713). However, analysis of perspiration is an analysis of the liquid of perspiration.

In addition to monitoring endogenously produced species, it is also possible to administer an isotopically tagged biomarker and detect one or more of the exhaled tagged metabolites. This procedure has been successfully utilized to elucidate metabolic pathways and detect disease. A case in point is the diagnosis of *Helicobacter pylori* infection in humans by exhaled air analysis. *Helicobacter pylori* is known to be the primary cause of chronic gastritis in humans. Although many persons infected with *Helicobacter pylori* do not demonstrate overt symptoms, the recent discovery of the *H Pylori* organism and its connection to disease of the upper gastrointestinal tract has resulted in major changes in thinking regarding the origin of gastrointestinal disorders.[11] Two exhaled air tests have been established for the diagnosis of *Helicobacter pylori* and include detection of either $^{13}CO_2$ or $^{14}CO_2$ resulting from the ingestion of tagged [C12, C13]urea.[12,13] Generally, exhaled air analysis occurs after preconditioning and/or preconcentrating the exhaled air. This may involve passing the exhaled air over a suitable desiccant, through a cold trap or mixing with an inert diluent to reduce water vapor. The sample may then be preconcentrated by bubbling through an appropriate solvent/indicator or condensed to remove only the "active" species. Detection of selected species is subsequently performed using either mass spectroscopy, scintillation spectroscopy, colormetric spectroscopy, gas chromatography and, more recently, gas phase infrared absorption spectroscopy.

Other methods of detecting *Helicobacter pylori* are based upon measuring the presence of ammonia produced. It has recently been discovered that an ammonium electrode can be used to indicate the presence of HP bacteria in gastric tissue. (See Butcher, et al., in Digestion, 1992, volume 53, pages 142 through 148). However, this discovery was of the use of such an electrode on in vitro (cell cultures in a laboratory) and not in vivo (in a living patient). Biopsies were required, and information was obtained only for the condition present at the time that the biopsy was obtained. No in patient, continuous, real time, ambulatory

monitoring was indicated, nor was the possibility of combining such measurements, with simultaneous measurement of other related parameters.

An in vivo method of measuring ammonia is reported in U.S. patent 5,477,854 issued Dec. 26, 1995 to Essen-Moller, Anders. Anders discloses a system and a method for in vivo monitoring intra-gastrointestinal concentrations of ammonium during prolonged periods, as an indicator of the presence and activity of an intragastrointestinal *Helicobacter pylori* and other ammonium producing infections. Ambulatory monitoring is possible with Anders' system. Anders uses an ambulatory digital recorder connected to an ammonium sensitive intragastrointestinal catheter and a reference Ag/AgCl catheter. After a calibration, the ammonium catheter is put into its intragastrointestinal position and the recorder samples, once per second, the values of ammonium concentration continuously measured by the ammonium catheter. After recording, the stored values are uploaded to a computer which analyses the ammonium data.

Uses of tunable infrared semiconductor lasers for exhaled air analysis have been reported in the literature by a number of researchers.[14-20] Advantages of laser absorption over more conventional techniques such as Fourier transform infrared (FTIR)[21] and non-dispersive[22] spectrscopies include higher sensitivity, greater spectral resolution, faster data acquisition and potentially compact packaging of the apparatus for field deployment. Gas phase infrared absorption spectroscopy has the further advantage of requiring minimal preconditioning and/or preconcentration of the sample prior to analysis. In their paper LASER BASED ANALYSIS OF CARBON ISOTOPE RATIOS, Science, vol. 263, 18 Feb. 1994, DE Murnick and BJ Peer use a CO_2 tunable laser to measure a ^{13}C tracer. Others have used lasers to measure isotopes of carbon, oxygen, nitrogen, hydrogen, carbon monoxide, nitrogen oxide, diatomic nitrogen and water vapor (U.S. patent 5,394,236). In addition, near infrared, room temperature lasers have been used to detect isotopes of carbon monoxide and carbon dioxide (U.S. patent 5,317,156).

It is recognized that distinguishing between isotopes, especially carbon isotopes is often difficult (Murnick and Peer). Obviously, the in-vivo measurement of ammonia is invasive and uncomfortable to the patient.

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SUMMARY OF THE INVENTION

The invention is a method of measuring ammonia in a breath sample with a laser. The invention is particularly useful for indicating the presence and activity of an intragastrointestinal *Helicobacter pylori* or other ammonia compound producing infections.

It is an object of the present invention to provide a non-invasive, accurate method of indicating the presence and/or activity of ammonia compound producing metabolic activity.

The subject matter of the present invention is particularly pointed out and distinctly claimed in the concluding portion of this specification. However, both the organization and method of operation, together with further advantages and

objects thereof, may best be understood by reference to the following description taken in connection with accompanying drawings wherein like reference characters refer to like elements.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a flow chart of a PBPK model.

FIG. 2 is a schematic of a laser system.

FIG. 3a is a near infrared spectrum of carbon dioxide and water.

FIG. 3b is a near infrared spectrum of carbon dioxide, water and ammonia.

FIG. 4 is a near infrared spectrum of $^{15}NH_3$ and $^{14}NH_3$.

FIG. 5 is a near infrared absorbance graph of ammonia from four samples.

DESCRIPTION OF THE PREFERRED EMBODIMENT(S)

The invention is a method of measuring ammonia in a breath sample with a laser. The invention is particularly useful for indicating the presence and activity of an intragastrointestinal *Helicobacter pylori* or other ammonia compound producing matabolisis.

Collection of a breath sample is done differently for exhaled air compared to perspiration vapor. For exhaled air, the exhaled air is collected directly through a small diameter tube. For perspiration vapor, the tube is placed on or near the skin to obtain a sample of the perspiration vapor that is in equilibrium or near equilibrium with the perspiration liquid.

Analysis of breath for the presence of chemical species indicative of specific diseases or as a means of following metabolic processes may be accomplished by (1) measuring absolute concentrations, (2) measuring altered ratios, or (3) measuring tagged species. In the third method of analysis, the tagged species is a biomarker that is preferably non-radioactive with a small natural abundance. In the present invention, isotopes of nitrogen and/or hydrogen are preferred as would

be present in an ammonia compound including $^{15}NH_3$, $^{14}NH_3$, N^2H_3, NH_4^+, $^{15}NH_4^+$, $^{14}N^2H_4^+$ and combinations thereof.

Helicobacter pylori bacteria are known to break urea down into CO_2 and NH_3. Hence, a large change in the amount of ammonia is indicative of the presence of *Helicobacter pylori*. According to the present invention, a laser is used to monitor breath samples for NH_3 before and after an oral dose of urea. The detection of the presence of *Helicobacter pylori* may be improved by tagging the urea with an isotope, preferably a non-radioactive isotope. In that case, the ratio of the tagged ammonia to the untagged ammonia indicates the presence of *Helicobacter pylori*.

A physiologically based pharmaokinetic (PBPK) model was also developed to characterize uptake and tissue retention of urea and ammonia both in the rat and in man. Data were used to develop a PBPK model for urea exposure and ammonia disposition as shown in FIG. 1. Arterial pathways **10** and veneous pathways **12** of an ammonia model **14** and a urea model **16** are shown. When a metabolically produced ammonia compound reaches the liver, it can be converted to urea or remain as the ammonia compound for transport throughout the body and elimination via the lung or skin. This metabolic conversion is indicated by the dashed line **18** connecting the two models. The urea is eliminated via the kidneys. It is understood that when metabolically produced ammonia compound reaches the liver, it can also be incorporated into proteins and/or amino acids. In the H. Pylori positive case, urea is converted to its components ammonia and carbon dioxide in the gastrointestinal tract.

A system schematic is shown in FIG. 2. The laser **100** may be any laser, but is preferably a room temperature gallium arsenide near infrared laser. A laser beam **102** is directed through a sample chamber **104** containing a breath sample. A detector **106** produces an electrical signal that is amplified and analyzed. A beam splitter **108** may be used to direct a portion of the laser beam **102** to a reference chamber **110** when comparison to a reference sample is desired.

In operation, a breath sample is continuously pulled through the sample chamber **104** for the duration of the measurement (about 10 seconds). No preconcentration is employed. Pretreatment, when used, consists of passing the breath sample through sodium hydroxide pellets to remove water and carbon dioxide. However, ammonia compounds spectra are distinguishable from carbon dioxide and water spectra. Pressure in the sample chamber **104** is preferably kept at less than about 50 Torr, more preferably less than about 30 Torr and most preferably about 20 Torr and regulated by throttling both the pump and inlet. The reduced pressure eliminates pressure broadening of the absorption features. As the sample is pulled through the sample chamber **104**, repeated laser scans (1000 scans/sec) are acquired via a 12-bit 1.25 MHz transient digitizer.

The resulting digital data is co-added by a micro-computer (not shown). Each laser scan starts at the same initial spectral frequency, lasts approximately 0.82 ms (i.e., 1024 points x 800 ns) and covers a spectral region of approximately 7500 MHz. In this manner, approximately 10,000 scans may be averaged in about 10 seconds. The initial spectral frequency will depend on the absorber (ammonia compound) of interest.

In the sample chamber **104**, it is preferred to have both a long optical path length and a reduced operating pressure obtainable by using a modified Herriott cell.[22] This sample chamber **104** has two concave, slightly astigmatic mirrors that are separated by a gap of about 550-mm. One of the mirrors is fastened to a kinematic mount that permits transitional and angular adjustments. The second mirror is fastened to a rigid base plate and the two mirrors are held apart by a rigid aluminum frame. A Pyrex tube surrounds the mirrors so that the mirrors and 3-liter volume between the mirrors can be evacuated and/or filled with a sample. The astigmatic mirrors are configured to allow the output of a laser (preferably a near infrared diode laser) to be reflected between the two mirrors 182 times, for a total optical path length of 100-meters. The astigmatic or modified design of the mirrors reduces fringing effects caused by feedback of the laser that is problematic to any multipass optical system.

The sample chamber **104** may be operated in one of two modes, either as (1) a sample-and-hold or as (2) a constant flow device. In the latter case, an aerodynamic inlet nozzle permits the cell to be operated at a steady-state pressure, when continuously pumped. In either case, the gas pressure in the cell should be kept slightly above about 20 Torr (i.e., about 25 Torr) needed to meet Ladenberg's criteria. By adjusting the inlet nozzle cross-section and selecting the appropriate pump size, it is possible to tailor the gas residence time and steady-state pressure in the cell. Equation (12) is general and relates the cell pressure to inlet area and pumping speed as a function of time.

$$P_{cell}(t) = \backslash|f(1,B)\ (P_{in}\cdot Q_{in} - \exp[A-\backslash|f(t\cdot B,V)] \qquad (12)$$

where $A=\ln(P_{in}\cdot Q_{in})$, $B=Q_{in}+Q_p$, P_{in} is the pressure at the inlet, V is the volume of the cell and Q_{in} and Q_p are the volume flow rates for the inlet aperture and pump, respectively. The volume flow rate is approximated by $Q_{in} = C_{in}\cdot Area(inlet)$ where,

$$C_{in} = R(\backslash|f(g\cdot R\cdot T,M)) \qquad (13)$$

and g, R and T are the conventional thermodynamic constants and M is the molecular weight of "air" (i.e., 29.2 g/mole). Preferably, the volume flow rate would be matched to the data acquisition rate.

High spectral brightness (0.1 mW) and narrow line widths (0.0003 cm^{-1}) make tunable lead-salt infrared diode lasers ideal light sources for detection of Doppler limited samples. Tunable diode lasers are commercially available and may be specified for operation in the 3 to 300 micron region. A specific tunable diode laser will typically lase ± 50 cm^{-1} from some specified frequency and may cover a number of different molecular species. In addition, a tunable diode laser may be modulated at high frequencies (e.g., several 100 MHz to 1 GHz) thereby permitting use of phase sensitive, ultra-low noise detection schemes. The major

disadvantage of the lead-salt tunable diode laser is the need for cryogenic operation that requires either a mechanical cooler or a small reservoir of liquid or solid cryogenic material.

When the laser **100** is a tunable lead salt diode laser, it is placed inside a temperature stabilized, cooled Dewar unit that maintains the temperature between 120 and 65 K. The entire system can be operated under the control of a micro-computer.

Depending on the spectral region of interest, either high speed indium-antimonide, InSb (3,000 to 1,800 cm^{-1}) or mercury-cadmium-telluride, MCT (1,800 to 500 cm^{-1}) detectors **106** are utilized. A matched preamplifier **112** is used to increase the signal output of the detectors **106** and delivers the signal to both an oscilloscope and transient digitizer (not shown). Both the oscilloscope and digitizer are triggered synchronously with the ramping of the diode laser **100**. The oscilloscope trace permits an operator to align and optimize the apparatus in real-time. To increase signal to noise ratio, it is preferred to use a lock-in amplifier **114** that admits only signals in phase with the dither **116** frequency.

When a ratio of isotopically tagged species are to be monitored (e.g., $^{14}NH_3$ vs. $^{15}NH_3$), the criteria for selecting transitions is somewhat different. The two absorption features, corresponding to the two isotopes must occur relatively near to one another ($Dn_o \leq 0.1$ cm^{-1}). In order to calculate absolute absorbance values and avoid problems associated with changes of laser power vs. frequency, a "zero" waveform is recorded and a "baseline" waveform is generated. The zero waveform consists of acquiring data with the laser **100** turned off. The baseline waveform is associated with the I_o of the spectral waveform and is created by fitting the data waveform to a polynomial. Points for the polynomial fit are selected so that absorption features are avoided. The zero waveform is first subtracted from the spectral waveform and this difference waveform is then divided by the baseline or I_o waveform. The resulting waveform, I/I_o related to transmittance and can be converted directly to absorbance either by taking the natural log or assuming the small number limit of $\ln[X]=1-X$. The individual

peaks are then fit to a Gaussian, Lorentzian or Voigt profile depending on the pressure region.

The theoretical absorbance limit of this apparatus is dictated by shot noise and predicted to be on the order of 10^{-7}. Sensitivity (on a routine basis) is approximately 10^{-4} absorbance units and limited by the presence of fine fringes traced to ´etaloning within our Herriot cell sample chamber **104**. In some cases, we can improve this sensitivity by a factor of 10 with use of optimal filtering which consists of convolving the normalized spectral data with an appropriate response function, determined *apriori*. Absorbance signals corresponding to hundreds of parts-per-trillion by volume of a specific absorber may then be observed. This is equivalent to a partial pressure of approximately 10^{-7} Torr of absorber in one atmosphere of sample.

Example 1

An experiment was performed to demonstrate the utility of the present invention, specifically to demonstrate the ability to distinguish ammonia from carbon dioxide and water. High resolution (75 MHz) spectrometers were used in combination with reduced pressure less than about 30 Torr for sample analysis. The results are shown in FIG. 3a and FIG 3b. FIG. 3a shows spectra for water **30** and carbon dioxide **32**. FIG. 3b includes spectra **34, 36** for ammonia. Although one portion of the ammonia spectra **34** is interfered with water spectra **30** the second portion of the ammonia spectra **36** is free of any interference permitting unambiguous detection of ammonia.

Example 2

A second experiment was performed to determine the ability to distinguish between ammonia compounds, specifically to distinguish between $^{14}NH_3$ and $^{15}NH_3$. Samples containing a separate concentration of each ammonia compound were prepared and analyzed as in Example 1. Results are shown in FIG. 4.

Peaks **40, 42, and 44** are for $^{14}NH_3$. Peaks **46, and 47** are for $^{15}NH_3$. Hence, ammonia compounds are clearly distinguishable.

Example 3

A third experiment was performed to ascertain the sensitivity of ammonia compound measurements. A sample of exhaled air, reference sample, perspiration vapor sample, and ambient air sample each containing $^{15}NH_3$ were exposed to a near infrared laser. Results are shown in FIG 5. Sample concentrations are distinguishable from 46 ppb to 150 ppb and quantifiable to 300 ppt.

Closure

While a preferred embodiment of the present invention has been shown and described, it will be apparent to those skilled in the art that many changes and modifications may be made without departing from the invention in its broader aspects. The appended claims are therefore intended to cover all such changes and modifications as fall within the true spirit and scope of the invention.

CLAIMS

We claim:

1. A method of indicating the presence of an ammonia compound in a breath sample, the method comprising the steps of:

(a) passing the breath sample into a reduced pressure chamber;

(b) directing a laser beam through the breath sample; and

(c) detecting the intensity of the laser light transmitted through the breath sample and obtaining a spectral response and determining a concentration of the ammonia compound in the breath sample.

2. The method as recited in claim 1, wherein a non-radioactive isotope is within a second ammonia compound that is used as a biomarker in the breath sample.

3. The method as recited in claim 2, wherein the non-radioactive isotope is a nitrogen isotope.

4. The method as recited in claim 3, wherein the nitrogen isotope is ^{15}N.

5. The method as recited in claim 1, wherein the reduced pressure is at least 1 Torr.

6. The method as recited in claim 5, wherein urea is administered to a patient and the breath sample is monitored for the presence of the ammonia compound indicating a breakdown of the urea.

7. The method as recited in claim 6, wherein the breakdown of urea is caused by *Helicobacter pylori*.

8. The method as recited in claim 1, wherein the breath sample is exhaled air from a lung.

9. The method as recited in claim 1, wherein the breath sample is perspiration vapor transpired through skin.

10. The method as recited in claim 1, wherein said laser has a wavelength from about 600 nm to about 1700 nm and a lase of about ±50 cm^{-1}±.

11. A method of detecting an infection of *Helicobacter pylori*, comprising the steps of:

(a) administering urea to a patient,

(b) obtaining at least one breath sample from the patient, and

(c) measuring an amount of an ammonia compound in said breath sample(s).

12. The method as recited in claim 11, wherein step (c) comprises,

(e) passing said breath sample(s) into a reduced pressure chamber,

(f) directing a laser beam through the reduced pressure breath sample(s),

(g) detecting laser light transmitted through the reduced pressure breath sample(s), and

(h) obtaining a spectral response of said ammonia compound.

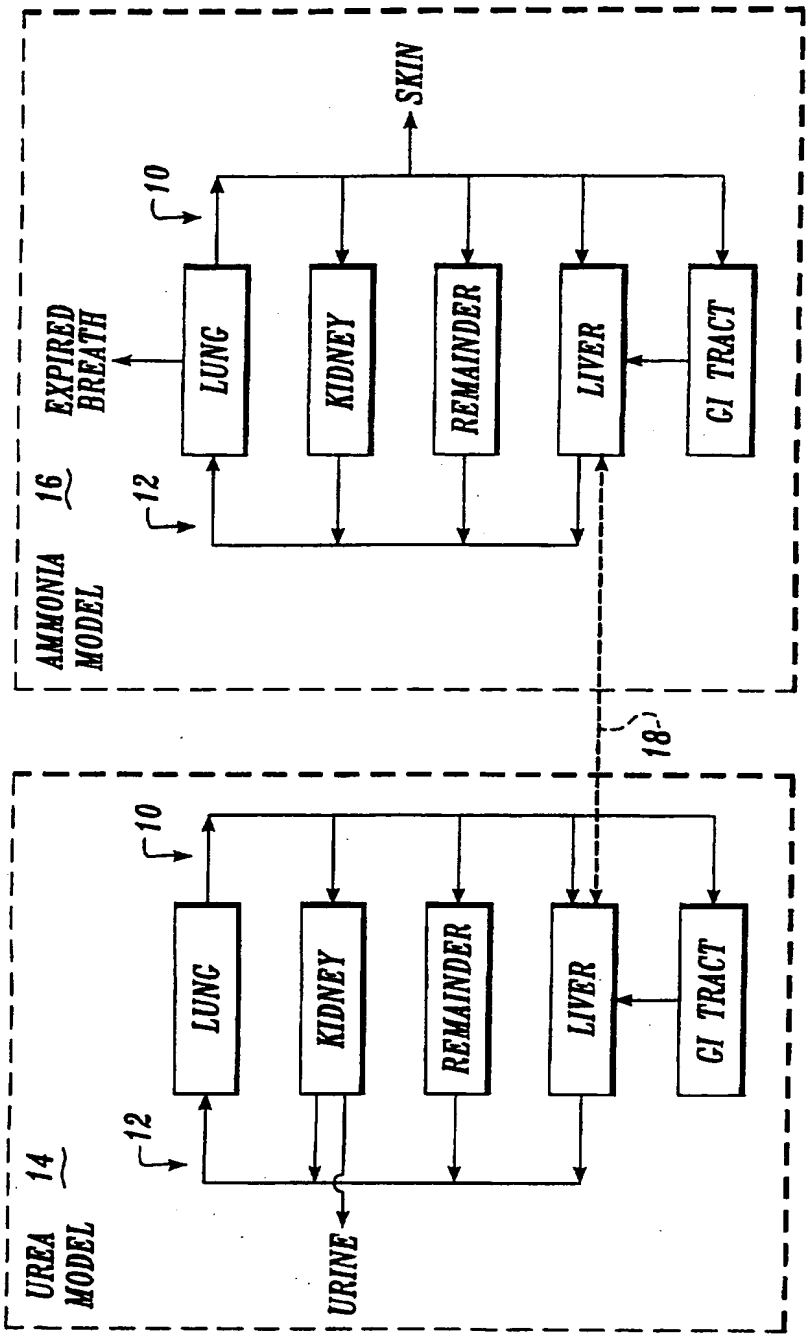
13. The method as recited in claim 12, wherein a non-radioactive isotope is within a second ammonia compound and is used as a biomarker in the breath sample.

14. The method as recited in claim 13, wherein the non-radioactive isotope is a nitrogen isotope.

15. The method as recited in claim 14, wherein the nitrogen isotope is ^{15}N.

16. The method as recited in claim 12, wherein the reduced pressure is at least 1 Torr.

17. The method as recited in claim 12, wherein said laser has a wavelength from about 600 nm to about 1700 nm and a lase of about ± 50 $cm^{-1}\pm$.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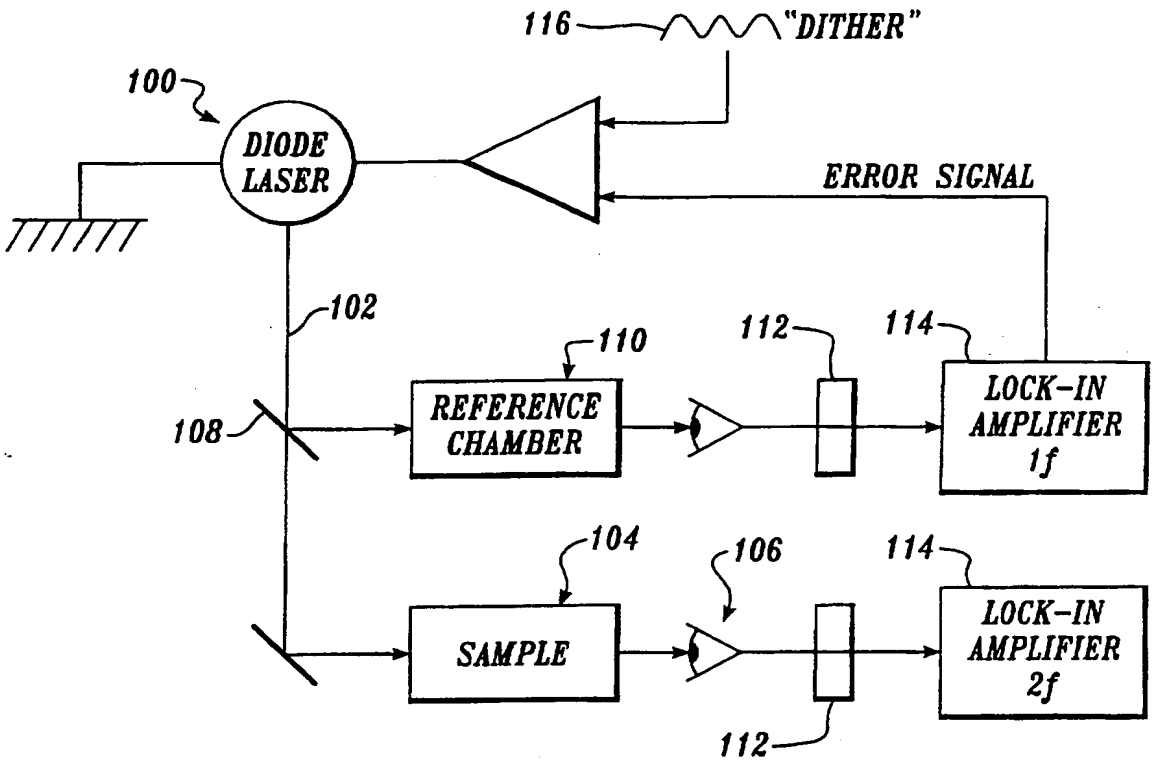
URINE

Fig. 1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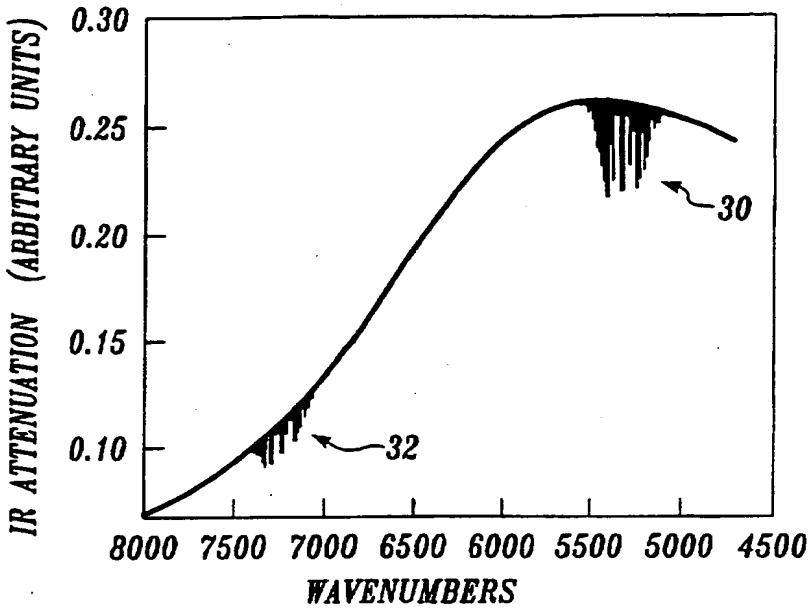
100
DIODE LASER
ERROR SIGNAL
102
110
112
114
108
REFERENCE CHAMBER
LOCK-IN AMPLIFIER 1f
104
106
114
SAMPLE
LOCK-IN AMPLIFIER 2f
112

Fig. 2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32
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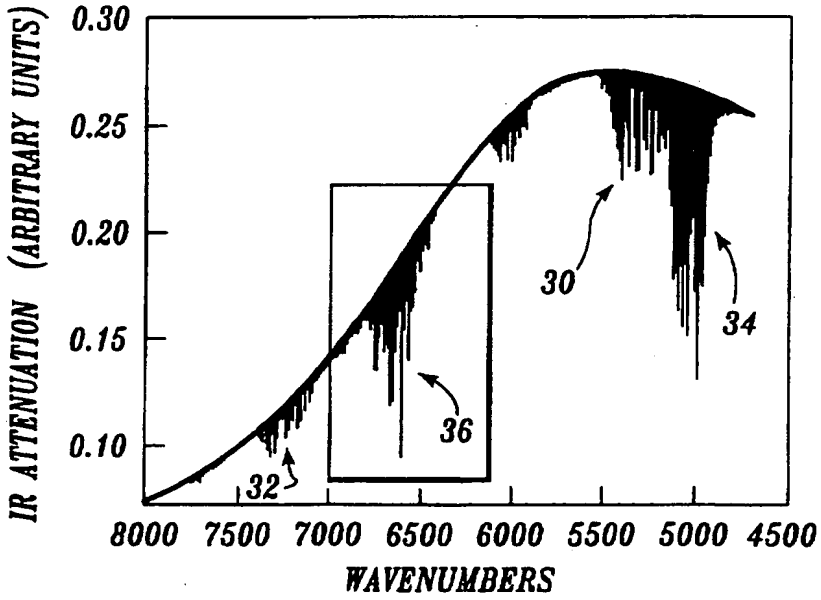
WAVENUMBERS

Fig. 3a


IR ATTENUATION (ARBITRARY UNITS)
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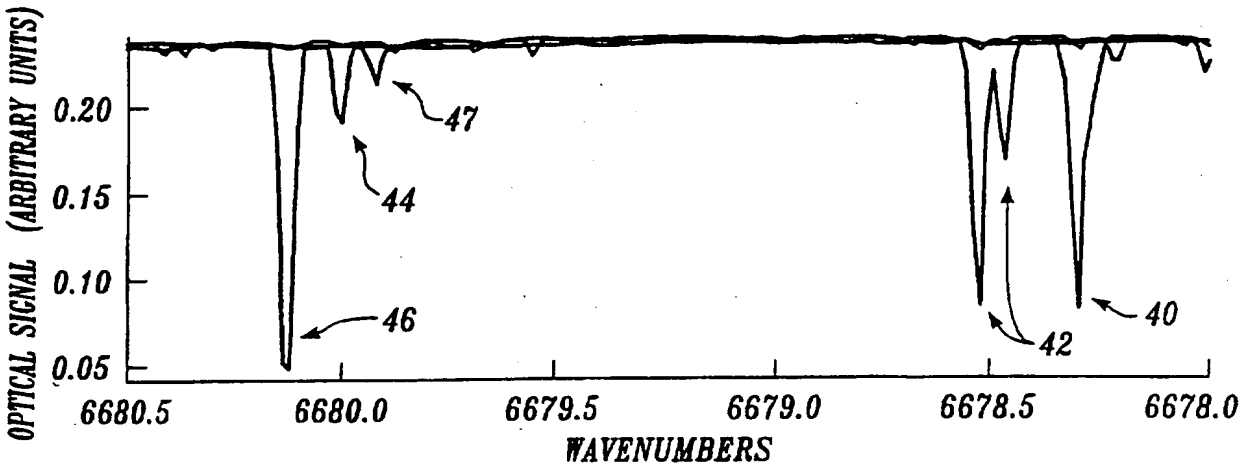
WAVENUMBERS

Fig. 3b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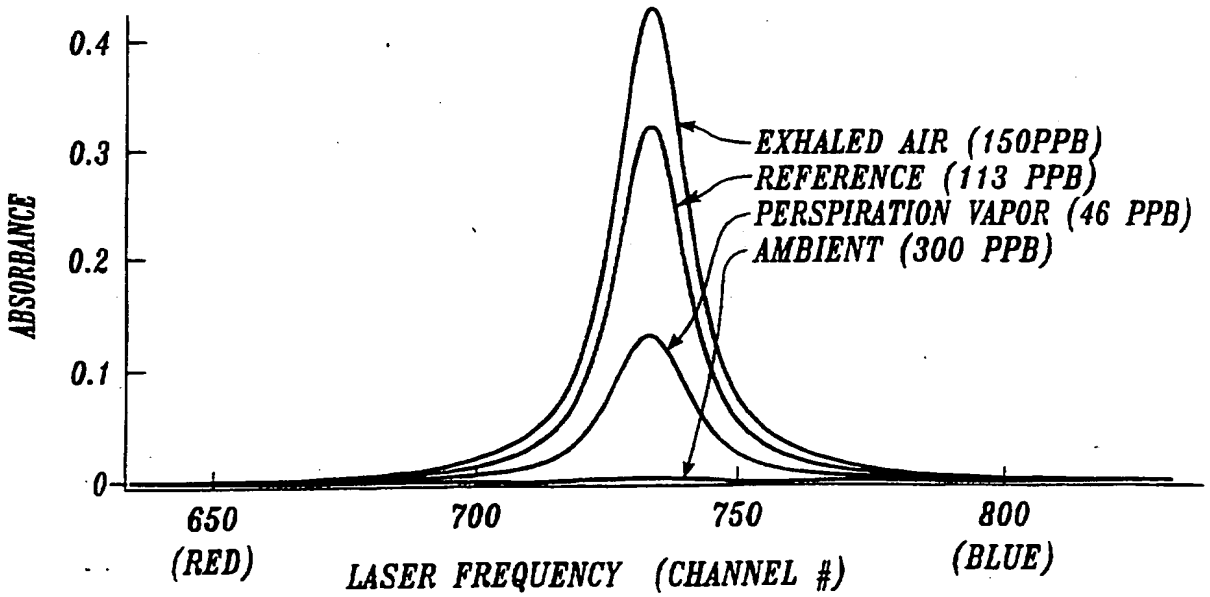
6678.5
6678.0
WAVENUMBERS

Fig. 4


ABSORBANCE
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0.3
0.2
0.1
0
EXHALED AIR (150PPB)
REFERENCE (113 PPB)
PERSPIRATION VAPOR (46 PPB)
AMBIENT (300 PPB)
650
(RED)
700
750
800
(BLUE)
LASER FREQUENCY (CHANNEL #)

Fig. 5

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 97/01126

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61B10/00 G01N21/35

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	REVIEW OF SCIENTIFIC INSTRUMENTS, vol. 58, no. 6, June 1987, NEW YORK US, pages 923-927, XP002031160 U. LACHISH ET AL: "Tunable diode laser based spectroscopic system for ammonia detection in human respiration" cited in the application see abstract see page 923, right-hand column, line 3 - line 8	1
Y	see page 926, right-hand column, last paragraph - page 927, left-hand column, line 18; figures 1,6	2,3,5-8, 10

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

3

Date of the actual completion of the international search	Date of mailing of the international search report
21 May 1997	06.06.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Thomas, R.M.

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INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/US 97/01126

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	PATENT ABSTRACTS OF JAPAN vol. 96, no. 4, 30 April 1996 & JP 07 323034 A (HITACHI), 12 December 1995, see abstract	11 12-14, 16,17
Y	US 5 394 236 A (MURNICK) 28 February 1995 cited in the application see column 1, line 42 - line 49 see column 4, line 65 - column 5, line 23 see column 8, line 37 - line 44 see column 9, line 47 - column 10, line 3 see column 15, line 64 - column 16, line 10; figure 1	2,3,5-8, 10, 12-14, 16,17
A	US 5 317 156 A (COOPER) 31 May 1994 cited in the application see column 1, line 6 - line 31 see column 4, line 49 - column 5, line 5 see column 8, line 8 - line 29; figure 2	1,5,6,8, 10,12, 16,17
A	THE LANCET, vol. 345, no. 8955, 15 April 1995, pages 961-962, XP000673516 S. KOLETZKO ET AL: "Isotope-selective non-dispersive infrared spectrometry for detection of Helicobacter pylori infection with 13C-urea breath test"	
A	ZEITSCHRIFT FÜR GASTROENTEROLOGIE, vol. 32, no. 12, December 1994, pages 675-678, XP000672026 B. BRADEN ET AL: "Clinically feasible stable isotope technique at a reasonable price ..."	
A	JOURNAL OF CLINICAL GASTROENTEROLOGY, vol. 20, no. S2, 1995, pages s115-s117, XP000672115 H. OHARA ET AL: "13C-UBT using an infrared spectrometer for detection of Helicobacter pylori and for monitoring the effects of lansoprazole"	

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte onal Application No
PCT/US 97/01126

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		AU 3211493 A	05-08-93
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		EP 0556614 A	25-08-93
		JP 7020054 A	24-01-95
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		JP 7503319 T	06-04-95
		WO 9315391 A	05-08-93